VOLUME 17 NUMBER 1

NUTRITION REVIEWS

January 1959

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Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

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Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

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NUTRITION REVIEWS

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NUTRITIONAL ASPECTS OF EGG YOLK

The association between human beings and avian eggs has been long, tasty and, until recent times, not too controversial. It might be expected, therefore, that nutritional aspects of egg yolk would be especially well elucidated. The fact is, this food is (1) poorly defined in chemical terms, (2) loosely used in many nutritional studies, and (3) remains an enigma to dietitians and physicians who often have practically no basis for knowing whether the inclusion of eggs in the diet of a given individual is beneficial to his health or contributory to his demise.

It has been known for some time that the yolk contains approximately 33 per cent lipid and 16 per cent protein. Until quite recently, however, only "ovovitellin" and "ovolivitin" had been isolated as proteins from the yolk (E. Chargaff, *J. Biol. Chem.* **142,** *491 (1942)*) and these are of such a vague character, chemically, that they constitute scarcely any advance in our knowledge of egg yolk. Within the last few years D. K. Meecham and H. S. Olcott (*J. Am. Chem. Soc.* **71,** *3670 (1949)*) have isolated a phosphoprotein from egg yolk which they have labeled "phosvitin" and other workers have promulgated various lipoproteins, in some cases, unfortunately, after lipid extraction! Histochemical studies (V. D. Marza and E. Marza, *Quart. J. Micr. Sci.* **78,** *134 (1935)*) have shown the presence of nucleoproteins in certain of the egg yolk granules.

Thus, it can be ascertained that much remains to be learned about the macromolecular constituents of egg yolk, constituents which have a decided nutritional importance. It is from this perspective that studies just completed in our laboratories may be of interest to nutritionists (O. A. Schjeide and M. R. Urist, Experimental Cell Research (*in press*)).

Using the ultracentrifuge for resolution and isolation, we have examined separately two major compartments of egg yolk, the yolk granules (comprising one-fifth of the total yolk volume) and the fluid in which the granules are suspended. Yolk granules are dissolved by the addition of 10 per cent of NaCl and upon centrifugal, electrophoretic and chemical analysis are found to consist of two main types of protein, a phosphoprotein comprising about 7 per cent of the total protein of egg yolk and a dense lipoprotein which makes up approximately 40 per cent of the total.

The phosphoprotein (which is identical to the "phosvitin" of Meecham and Olcott (*loc. cit.*)) consists of a high proportion of serine containing phosphorus in an o-ester linkage. According to our calculations, this amino acid may comprise as much as one-fourth of the total amino acids in the phosphoprotein. Its molecular weight has been calculated roughly to be 77,000. It holds approximately 75 per cent of the calcium of egg yolk, thus exhibiting a ten times greater capacity for binding calcium than does casein of milk. (The total calcium of egg yolk is 100 mg. per cent. M. R. Urist, O. A. Schjeide, and McLean (*Endocrinol.* **63,** *570, 1958*))

The dense lipoprotein contains about 20 per cent lipid of which 80 per cent is phospholipid. It also contains a significant amount of protein bound polysaccharide and is considered to be a glycolipoprotein. Although considerable sulfur is included in this molecule, it is not known how much is in the amino acid form and how much may be present in the polysaccharide moiety. (About half of the sulfur present in granules is not protein bound.) The molecular weight of the dense lipoprotein has been calculated to be of the order of 200,000. It does not normally bind appreciable amounts of calcium. Both

the dense lipoprotein and the phosphoprotein, described in the preceding paragraph, are synthesized in the liver of the hen, and are carried through the blood to the ovaries, where they are passed (with some modification) through the egg cell membranes to become solid structures of the yolk.

At least two other proteins are present in the yolk granules but in much lesser concentrations. One of these is a nucleoprotein and the remainder consists of low density lipoprotein, containing mainly triglyceride as the lipid component.

The phosphoprotein and dense lipoprotein, which comprise the major portions of the egg yolk granules, are not detectable in the other large compartment of egg yolk, the egg yolk fluid. Chief macromolecular constituents of this compartment include a substance tentatively identified as "ovalbumin," although it may differ in some respects from the ovalbumin of the egg white, and some low density lipoproteins which float as a fairly discrete grouping at S_f 21. The lipid moieties of the latter make up about 80 per cent of the molecules and are largely triglyceride. Over 50 per cent by weight of the yolk fluid is lipid in this lipoprotein form. Fifteen per cent of the total calcium in egg yolk is bound to the proteins of egg yolk fluid.

Although these studies have barely extended the frontiers of our knowledge of egg yolk and its proteins, one obvious conclusion can be drawn: the use of whole egg or egg yolk as "reference proteins" for determination of relative nutritional values of various plant and animal proteins, constitutes a questionable procedure. Even if yolk alone is the standard, comparison is being made with a material which contains, at the very least, twice as much lipid (protein bound) as it does protein and which is composed of not one or two but several distinctly different proteins. It would seem that high priority should be given to a re-examination of the growth supporting properties of egg yolk in which each macromolecular entity is tested separately and in various combinations with other yolk and egg white constituents. In terms of identity, superior reference proteins may emerge from such screening.

There is insufficient correlative evidence in the literature to permit the prediction that any one of the proteins present in egg yolk should be especially conducive to growth. The fact that yolk proteins, as a group, are particularly rich in the essential amino acids (*Nutrition Reviews* **15,** *151 (1957)*), does lead to the exciting speculation that one of them may contain a proportion of such acids which exceeds the mean. But purely protein considerations may be obscured by the action of protein bound substances other than amino acids, such as lipids, carbohydrates, minerals, hormones, vitamins and purine and pyrimidine bases.

It is also possible that requirements for growth of the young animal or child are met unequally by the yolk proteins because of differences attributable to the specific nature of the growth taking place. During the course of embryonic development, the organism is exposed to differing proportions of egg yolk proteins. In the initial phases of development and growth it is bathed in yolk fluid which contains, along with free carbohydrate (chiefly as glucose), those proteins to which we have referred as ovalbumin and which are glycoproteins. Only during the middle and later phases of growth are the yolk granules dissolved, and the lipoproteins utilized to any great extent. When dissolution of the yolk platelets in embryo frogs takes place, we note quick utilization of the phosphoprotein component whereas the dense lipoprotein (minus a portion of its lipid complement) is still detectable in extracts of tadpoles of the tailbud stage (O. A. Schjeide, E. Levi, and R. A. Flickinger, *Growth* **19,** *297 (1955)*).

We are not, however, solely concerned with growth promoting aspects of egg yolk. In the United States, as in most countries, more eggs are consumed by adults than by children. W. Lintzel (*Deutsche med. Wochenschrift* **80,** *1047 (1955)*) and others

have raised the issue of "maintenance proteins" versus "growth promoting proteins" in maintaining the nitrogen balance and have claimed that proteins from plants are most efficient with respect to maintenance and that animal proteins, including egg proteins, are essential only for growth. Although Lintzel's conclusions have been criticized and, in fact, convincingly disproved (E. Kofranyi, *Z. F. Physiol. Chem.* **309,** *253 (1957)*), it seems reasonable that an adult would require less than a child in the way of growth promoting protein, such as that found in eggs. However, it would seem that special attention should be given to the elucidation of the roles of isolated yolk constituents in such adult-type and nutritionally influenced diseases as arteriosclerosis.

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GENETIC ASPECTS OF METABOLIC DISEASE IN CHILDHOOD—PART II

By far the greatest number of congenital diseases encountered are due to defects in protein synthesis (D. Gitlin and C. A. Janeway, *Pediatrics* **21,** *1034 (1958)*). Defects in the synthesis of specific enzymes are usually considered under the specific actions of these enzymes. An example of a specific defect in protein synthesis is congenital afibrinogenemia, a bleeding disease characterized by absence of fibrinogen from the blood, which completely fails to clot. Although only a handful of cases have been reported these have occurred in families in which there is a high incidence of blood relationship suggesting that it is a recessive characteristic. Using sensitive immunochemical methods Gitlin and Janeway have shown that the distribution of fibrinogen within the body, and its turnover rate, were normal and that the essential defect was inadequate synthesis of this specific protein. Hemophilia, involving an inability to synthesize one of the other factors necessary in blood clotting, is another well known genetic disease. Indeed, the congenital absence of almost all of the known factors involved in coagulation has been found.

Agammaglobulinemia is manifested by absence of the gamma globulins, the fraction of the serum proteins containing bacterial antibodies. The congenital form appears to be a sex-linked recessive genetically, and the involved children suffer from recurrent severe bacterial infections, such as pneumonia, meningitis or septicemia. Studies by Gitlin and Janeway have also shown this defect to be due to inadequate rates of synthesis of gamma globulin by the plasma cells in the lymphoid tissue.

A defect in the synthesis of one of the more specialized serum proteins, ceruloplasmin, which is generally known as the copper carrying protein, may prove to be the basic defect in Wilson's disease, or hepatolenticular degeneration. Present evidence would suggest that the defective synthesis of ceruloplasmin may be indirectly associated with copper metabolism in these patients (*Nutrition Reviews* **16,** *36 (1958)*), giving rise to increased tissue stores of copper and altered function of the basal nuclei of the brain, liver and kidneys.

Included under defects in protein synthesis are the diseases associated with abnormal hemoglobins. Since the initial observations that the hemoglobin in sickle cell disease had a different electrophoretic mobility than that of normal adult hemoglobin, some 12 or 13 different abnormal hemoglobins have been described, each having its own pattern of association with various ethnic groups.

Another area of congenital metabolic

abnormalities is that involving the reabsorption of amino acids and other substances by the renal tubules (C. U. Lowe, *Pediatrics* **21,** *1039 (1958)*). A number of syndromes have been described in the past in which an abnormal amount of organic acid appeared in the urine. Amino acids constitute the major portion of organic acids, but other organic acids probably are present as well. They have been less well characterized because methods for their detection have not been available. The syndromes including aminoaciduria are divided into two groups, those in which the blood concentration of the amino acids is elevated and those in which it is normal. Phenylketonuria is about the only well known anomaly which falls in the first group.

The diseases in which aminoaciduria is secondary to a failure of the renal tubule to reabsorb amino acids from the glomerular filtrate are divided by Lowe into four groups: those due to toxic damage, such as lead poisoning, irradiation, severe burns, hypervitaminosis, or lipoid nephrosis; genetic defects producing a toxic substance such as copper in Wilson's disease or galactose in galactosemia; outright deficiency disease, such as is seen in vitamin D deficient rickets; and, lastly, genetic defects. The spectrum of the aminoacidurias also has been reviewed recently by H. E. Harrison and H. C. Harrison, (*J. Am. Med. Assn.* **164,** *1571 (1957)*; (*Nutrition Reviews* **16,** *39 (1958)*).

Among the genetic diseases, cystine-lysinuria is characterized by the appearance in the urine of abnormally large amounts of cystine, lysine and ornithine. This abnormality is entirely renal in origin and is due to impaired tubular reabsorption of the involved amino acids. The clearance of cystine is equal to the glomerular filtration rate. This syndrome had previously been known as cystinuria, because the insoluble nature of cystine and the formation of renal stones is one of the prominent clinical manifestations. Cystine-lysinuria is a completely separate disease from cystinosis or cystine storage disease, with which it has been partially confused. Another entity is the H. syndrome, or Hartnup syndrome, in which several amino acids are present in the urine. These patients have episodes of clouded sensorium, cerebellar ataxia, and a rash, strangely like that occurring in pellagra, is also present. These manifestations occur sporadically and are not associated with any changes in the degree of aminoaciduria.

Under the heading of infantile De Toni syndrome, Lowe refers to those cases characterized by rickets, dwarfism, hypophosphatemia, hyperphosphaturia, glycosuria and, frequently, aminoaciduria. Cystine storage has not been found in most of the patients having this syndrome in early life and the course of the illness would suggest that, although vitamin D-resistant rickets is part of the picture, the severe renal involvement clearly separates these patients from uncomplicated vitamin D-resistant rickets. Lowe considers Milkman's syndrome to be an adult form of the same illness with osteomalacia and glucosuria, although no record of organic acid determinations in the urine of these adult patients has been made. Many other authors, however, feel that cystine storage is an integral part of the De-Toni-Fanconi-Debré syndrome (H. G. Worthen and R. H. Good, *Am. J. Dis. Child.* **95,** *653 (1958)*).

The finding of aminoaciduria in patients with vitamin D resistant rickets, as well as in those with vitamin D deficient rickets, would fit in well with the previous observations that phosphate and glycine, the most prominent amino acid in both these conditions, share a common transfer mechanism in the renal tubule. The suggestion that vitamin D resistant rickets results from refractoriness of the end organ to an amount of vitamin D sufficient in normal individuals has been advanced. Another organic aciduria is unique in that it is characterized by a specific anatomic abnormality, as well as the renal defect. Children with buphthalmos and organic aciduria are born with glaucoma, severe mental retardation, systemic acidosis, and an organic aciduria in which only a

fraction of the organic acids can be accounted for as amino acids.

Summary. Thus, genetic defects interfering with the normal metabolism of a number of nutrients result in well defined diseases. The nutrients concerned are proteins, amino acids, sugars, and complex metabolites derived from these simple substances. In a number of instances the resulting toxic substance or a deficiency secondary to the genetic defect in metabolism may be corrected by dietary means. In other instances, we have a better understanding of the defect in the metabolic process although no specific therapy is possible at the present time. The concept of the genetic basis for enzyme deficiencies will undoubtedly be responsible, in the future, for a better understanding of many metabolic diseases. Their treatment is a challenge to the science of nutrition.

JEJUNAL LESIONS IN SPRUE

No agreement of opinion exists as to the nature, or even presence, of anatomic changes in the small intestine of patients with sprue. This is due largely to the paucity and poor quality of pathological material. Cases in any one series are few, and autopsies are fewer. Post-mortem studies, as in other tropical diseases, are often vitiated by the lack of good laboratories and, especially, by post-mortem autolysis. G. E. Butterworth, Jr. and E. Perez-Santiago (*Ann. Int. Med.* **48,** *8 (1958)*) have published an article entitled "Jejunal Biopsies in Sprue", from the U. S. Army Tropical Research Medical Laboratory in San Juan, Puerto Rico.

Although histologic changes had been noted by various authors as early as 1890, several observers could see no abnormality (in autopsied material) and the belief spread that the small intestine is histologically normal in sprue. Butterworth and Perez-Santiago give credit to E. Koppisch's study of 16 sprue autopsies in 1947 and J. W. Paulley's report in 1954 on jejunal biopsies from two cases of steatorrhea. Both observed changes similar to those to be described.

The present paper reports on jejunal biopsies in six patients "with the clinical features of sprue and laboratory-proved malabsorption." The specimens were obtained at laparotomy for unrelated causes, five of them from women undergoing bilateral tubal ligation. One had had transfusions but no specific therapy; the other four had received folacin "at some time." One patient was a male who also had lymph node and jejunal biopsies "to exclude Whipple's disease." Laboratory diagnosis rested on the urinary xylose excretion test after an oral dose, serum vitamin A level after an oral dose, serum turbidity after eating butter, and 24-hour fecal fat determinations. Fifteen control biopsies were studied from surgical patients who had no history of chronic diarrhea or anemia. One was a patient who developed diarrhea and megaloblastic anemia after delivery; she had normal absorption tests.

The authors describe the histologic details in the normal specimens, with photomicrographs, pointing out the long slender villi, scattered infiltrating cells and the tall, regular, columnar epithelium. Six case histories are given, plus that of the control with megaloblastic anemia. The histologic changes observed in the biopsies from patients with sprue may be summarized as follows: villi were generally shortened, widened, and appeared to coalesce with those adjacent. The tips were often described as wide, broadened, or "clubbed" and fused to adjacent tips producing "bridging." The authors calculated roughly the absorptive surface, from enlarged photomicrographs, and found that the "surface area of the normal was about four times greater than in the untreated sprue subject."

A second finding was inflammation of the lamina propria, or connective tissue of the mucosa. Cells, especially eosinophils and plasma cells, "greatly exceeded" the numbers normally found. Atrophy of the lamina propria was slight or absent, and the authors wonder whether malnutrition and gaseous distension at autopsy may have accounted for the observed thinning in older reports. They describe edema in the lamina propria of every case, extending to the submucosa in some.

Thirdly, there were pronounced and consistent changes in the epithelium. The mucus-secreting goblet cells were increased in several cases. The columnar epithelial cells were flattened, markedly vacuolated, nuclei were few and nuclear debris was present. "Here the normal columnar layer with abundant cytoplasm and nuclei in palisades have given way to a thin shell of cell remnants." The changes in the photomicrographs are striking indeed. The contents of the vacuoles were not identified; in the one specimen stained for fat they did not take the stain. Nor could glycoprotein be demonstrated, as in Whipple's disease. The epithelial changes were noted in various degrees in all sprue cases except one. "This man differed from all the others in that he had never been anemic and did not have megaloblastic marrow changes in spite of unquestionable malabsorption." No consistent changes were found in the submucosa and muscularis. The authors did not mention the neurenteric plexuses, and presumably did not specially study them.

Butterworth and Perez-Santiago dissociate their findings from the epithelial changes and atrophy of the intestine reported in autopsy material on starvation, pointing out that none of their sprue patients had severe malnutrition. Likewise the glycoprotein-containing foam cells in the intestinal mucosa in Whipple's disease (intestinal lipodystrophy) distinguished this disease of malabsorption.

The authors point out that the reduction in absorptive surface can scarcely be the cause of the deficient absorption in sprue, though it probably aggravates matters. Indeed, patients have maintained their nutriture after surgical removal of up to 90 per cent of the small intestine.

It seems possible, or likely, that the fusion of the villous tips represents the end result of the healing process, following micro-ulceration of villi whose denuded surfaces might then adhere permanently during repair. The inflammatory cells in the mucosa are consistent with this. Butterworth and Perez-Santiago point out the rapid rate of replacement of intestinal epithelium normally (perhaps two or three days in irradiated laboratory animals) and draw attention to the striking improvement of many patients within one or two days of folacin or vitamin B_{12} treatment. The cytologic changes of stomach epithelium reported in sprue and pernicious anemia support the thesis that folacin or cyanocobalamin deficiencies manifest themselves chiefly in damaged nuclear development in the most rapidly reproducing cells, *e.g.* bone marrow and gastrointestinal epithelium. The authors report appreciable enlargement of nuclei in cells of the buccal mucosa of sprue patients, stained by the Papanicolaou technique. They conclude, referring to the jejunum, that "either diminished production or shortened survival of columnar cells could, by jeopardizing the integrity of this surface, lead to all the changes noted."

Although the pathologic changes described by Butterworth and Perez-Santiago are probably effects rather than causes of sprue, and may not even be specific, the paper is valuable because it establishes objective changes in sprue and explains the apparent contradictions inherent in much previous work, based as it was on postmortem material with varying degrees of autolysis.

Findings very similar to those reviewed here are reported by R. J. Bolt, H. M. Pollard and L. Standaert in an article entitled

"Transoral small-bowel biopsy as an aid in the diagnosis of malabsorption states" (*New Engl. J. Med.* **259,** *32 (1958)*). These investigators used a suction-guillotine biopsy tube passed, by mouth, into the proximal jejunum. Adequate specimens were obtained in 30 of 34 patients. Seven patients had "characteristic" clinical and laboratory findings of "nontropical sprue or 'idiopathic steatorrhea' ". All showed similar histologic abnormalities: mucosal "atrophy, cellular infiltration of the tunica propria, flattening of the epithelial surface of the villi and very obvious coalescence of the villi." The authors consider the changes diagnostic of nontropical sprue. Their low power photomicrograph is quite similar to those of Butterworth and Perez-Santiago, but epithelial changes are not described. A similar lesion is pictured in the case records of the Massachusetts General Hospital (Case 44362, *New Engl. J. Med.* **259**, *431, 475 (1958)*).

In summary, jejunal biopsies from six patients with sprue showed flattening of the columnar epithelium with vacuolation and fragmentation. Chronic inflammatory cells were abundant in the mucosa; the villi were shortened and widened, with fusion of the tips. It is suggested that these changes are the result of widespread damage of the gastrointestinal epithelium, perhaps by folacin deficiency. Transoral biopsies in another report showed similar changes in nontropical sprue. Failure of previous studies to show lesions consistently is ascribed to the autolysis of post-mortem material.

IRON DEFICIENCY FOLLOWING SUBTOTAL GASTRIC RESECTION

Surgical removal of 60 per cent or more of the stomach for the treatment of a gastric or duodenal ulcer is a method of combating this disorder. By such an operation the rate of ulcer recurrence has been reduced to approximately 4 per cent; however, in recent years evidence has been accumulated that certain metabolic abnormalities may occur following interruption of the normal continuity of the gastrointestinal tract (*Nutrition Reviews* **15,** *260 (1957)*; **15,** *248 (1957)*).

Following a gastric resection, the continuity of the gastrointestinal tract can be re-established by anastomosing the cut end of the stomach with the small intestine in one of two general ways. One operative technique, which is called the Billroth I, connects the duodenum with the cut end of the stomach. When the Billroth II type of operation is employed, the stomach remnant is joined to the jejunum distal to the ligament of Treitz, thus producing a blind pouch at the duodenum. R. M. Zollinger, and E. H. Ellison, (*J. Am. Med. Assn.* **154,** *811 (1954)*) suggested that nutritional disturbances in patients following a gastric resection of the Billroth II type are more frequent than in those after the Billroth I.

The making available, nutritionally, of the naturally occurring iron compounds in foods is aided by gastric hydrochloric acid, but the gastric resection is designed specifically to reduce the concentration of this acid. Thus, derangements in iron metabolism might be expected following such a surgical procedure. The chief site of iron absorption occurs in the first portion of the duodenum, and beyond this the absorptive capacity of the intestine for iron decreases. In the case of the gastrojejunostomy, because of the re-routing of the stomach contents, it is possible for the ingested foods to by-pass the entire duodenum, as well as a small portion of the proximal jejunum. Recently, J. M. Rumball and C. P. Hassett (*Gastroenterology* **32,** *887 (1957)*) have studied the hemoglobin, hematocrit and serum iron levels of 125 patients who had subtotal gastric resections of the Billroth II type (gastrojejunostomy) performed during the

period of 1947 to 1954. Among these patients were 13 individuals selected because they presented gastrointestinal complaints following the operation. All patients had their operations performed at least one year prior to this study.

Blood studies were carried out on 25 normal control individuals (non-gastrectomized) and it was found that they had an average hemoglobin level of 14.6 g. (13.0 to 16.6) per 100 ml., an average hematocrit of 47 per cent (43 to 52), and an average serum iron level of 108.4 micrograms (70 to 170) per 100 ml. In handling the data these investigators divided the 125 subtotally gastrectomized patients into four groups, according to their status of iron metabolism.

The first group consisted of 82 patients whose hemoglobin, hematocrit, and serum iron levels were within the normal range (12.0 to 17.2 g. per 100 ml., 40 to 54 per cent, and 80 to 250 micrograms per 100 ml., respectively). It was noted that five subjects in this group had serum iron levels above 170 micrograms per 100 ml. The cause of this was not investigated. The second group consisted of 11 patients who had borderline hypoferremia. They had normal hemoglobin levels ranging between 12 to 16.1 g. per 100 ml., hematocrits between 40 and 54 per cent, but low serum iron levels of 70 micrograms per 100 ml. (no range was given). The third group of patients had hypoferremia without anemia, and this group consisted of 18 patients whose hemoglobin, hematocrit and serum iron levels ranged between 12 and 16.1 g. per 100 ml., 40 to 51 per cent, and 20 to 60 micrograms per 100 ml., respectively. The fourth group of patients (14) had hypoferremia with frank anemia. They had an average hemoglobin content of 9.6 g. (5.5 to 11.1) per 100 ml., a hematocrit of 35 per cent (23 to 39), and serum iron levels of 33.2 micrograms per cent (10 to 60).

The authors thought that the third group of patients presented interesting data in that their hemoglobin and hematocrit values were normal while the serum iron levels were definitely low. Some of these patients who were given oral iron therapy showed a slow response to this medication. On carrying out iron tolerance tests it was found that these individuals did not show a large affinity for iron ("iron-hungry") which occurs in anemias caused primarily by blood loss iron deficiencies. On the other hand, the patients in group four who had low hematocrit, hemoglobin and serum iron levels showed a large affinity for iron with a rapid increase in serum iron levels after iron administration. Even though no definite lesions which might have induced a blood loss could be found in this group of anemic patients, the iron tolerance curve indicated that iron deficiency anemia was of the blood loss type. Also, the patient's response to iron therapy was rapid, which would support this contention.

The question arises whether or not faulty diet, with or without malabsorption of nutrients, could produce an iron deficiency. C. V. Moore (*Am. J. Clin. Nutrition* **3,** *3 (1955)*) suggests that iron deficiency anemia rarely, if ever, is caused solely by a poor nutritional intake. Also, he noted that there is no published report in this country of iron deficiency anemia in the absence of blood loss. By determining the iron absorption of an adult male using radioactive iron he found that about 1.2 to 1.5 mg. of iron are absorbed each day on an intake of 10 to 15 mg. Since such an individual excretes 1 mg. or less of iron per day he maintained a positive balance. Theoretically, it would take a male six years to develop a definite iron deficiency anemia with a hemoglobin of 7.5 g. per 100 ml. if no iron was absorbed from his diet. Thus bleeding must be a major factor in the etiology of iron deficiency anemia according to this investigator.

Although the work of Moore and his associates is quite definitive in the case of normal individuals, there appear to be

little or no data in regard to the iron absorption or excretion by patients following a subtotal gastric resection. Until such data are obtained the course of the anemia in this group of individuals cannot be decided upon. Also, rational therapy for such an anemia cannot be provided until its cause is known. It would have been of interest had these investigators noted other nutritionally dependent factors, such as weight change and plasma protein levels, and the presence or absence of symptoms which might influence dietary intake along with indices of iron metabolism.

VASCULAR COMPLICATIONS IN PANCREATIC DIABETES

The etiological relationship of vascular complications in patients with diabetes mellitus to the disease itself has been the subject of considerable interest and discussion. The use of insulin therapy has reduced the severe complications of diabetic coma so that very few diabetic patients now die from coma and acidosis. In fact, the life expectancy of the average patient with diabetes, as indicated by insurance company statistics, is only a few years shorter than that of the general population.

Nevertheless, the majority sooner or later develop one or more of the so called degenerative changes associated with the disease. The incidence of vascular lesions in the retina and renal glomeruli of patients with diabetes is generally considered specific for this disorder. In fact, because of their frequency, some investigators consider them an integral part of the disease rather than a complication. P. M. Le Compte (*Diabetes* **6,** *180 (1957)*) has suggested that there is little evidence contradicting the view that the vascular complications of diabetes mellitus may be determined by the same gene which is responsible for the diabetes mellitus, since there are very few cases reported in which capillary lesions occur due to other known causes such as pancreatitis or pancreatic fibrosis.

Recently, L. J. P. Duncan, A. MacFarlane and J. S. Robson (*Lancet* **I,** *822 (1958)*) have reported a case of vascular disease involving both the eyegrounds and the kidney in a patient with diabetes due to chronic pancreatitis. They studied a 48-year-old man who developed mumps at the age of 11, and, after a few years, experienced episodes of vague abdominal discomfort. Eight years later, when he was 19, he developed the classical symptoms of diabetes mellitus. He had glycosuria and was treated by dietary restrictions and the administration of insulin. Sixteen years after having mumps he developed uremia.

In 1957, because of incontinence and recurrent infections of the kidney, the ureters were transplanted into an isolated loop of ileum which acted as a reservoir. In spite of therapy, the patient died within a few months of a urinary tract infection, toxemia and uremia. At the time of death he had moderately severe diabetic retinopathy.

This patient was shown to have inadequate pancreatic exocrine activity. By means of fat balance studies he was found to be absorbing only 60 per cent of the ingested fat. The large amount of fecal nitrogen, 6.6 g. per day, indicated that he also had poor absorption of protein. An analysis of duodenal aspirations, following an injection of secretin and pancrozymin, confirmed the virtual absence of exocrine pancreatic function since there was no response in pH, or in the flow of bicarbonate, amylase, trypsin or lipase.

On autopsy the pancreas consisted mainly of fibrous tissue with nodules of calcium. Very little true pancreatic tissue could be identified. The islet tissue was greatly reduced, with only small isolated groups of islets being found in fibrous tissue; none of these showed evidence of fibrosis, hyaliniza-

tion or any other lesions which could be associated with diabetes mellitus. The ratio of alpha to beta cells using the Gomori stain was ascertained to be normal. The kidneys of this patient showed very extensive pyelonephritic scarring and many of the glomeruli showed diffuse intercapillary sclerosis. A few contained small but typical nodule lesions of classical diabetic glomerulosclerosis.

Thus, a patient who had an abnormal carbohydrate metabolism due to a decrease in insulin content of the pancreas showed changes in both the eyegrounds and the kidney which were considered etiopathic of diabetes mellitus. This patient had no family history of diabetes. His parents died in their seventies. None of the parents or siblings had diabetes. He had two older brothers alive and well, and both they and the patient were found to have normal weights at birth.

The predisposition to diabetes mellitus may be inherited as a recessive characteristic, manifesting itself clinically in only a portion of those bearing the gene. The tendency to develop the specific vascular lesions could be inherited similarly, but the fact that these abnormalities have not been found with certainty in persons having no demonstrable defect in carbohydrate metabolism makes it improbable that they are inherited as independent diatheses.

Hyperglycemia, impaired glucose tolerance and the tendency to develop ketosis can arise in man as a result of disease of the pancreas. The development of specific retinopathy or nephropathy in such "pancreatic diabetics" would support the view that these complications result from abnormal carbohydrate metabolism in diabetes rather than as a consequence of an inherited defect. Duncan and associates have so far found only two previous cases in the literature in which patients developed vascular diseases without having known diabetes mellitus.

Until more information is accumulated concerning the intimate relationship of carbohydrate metabolism and the long term complications of diabetes (*e.g.*, vascular changes), the problem will remain unsolved. Nevertheless, these investigators have shown that the development of vascular changes in a patient with diabetes of pancreatic origin adds very strong evidence for the view that, to a large extent, vascular lesions are a consequence of a metabolic disorder resulting from the insulin deficiency rather than from a poor, inherited genetic constitution.

MEASURING FAT ABSORPTION IN INFANTS AND CHILDREN

During the last few years, study of fat absorption using I^{131} labeled fats has proven to be a useful technique for clinical investigation (*Nutrition Reviews* **15,** *171 (1957)*). A similar technique has been used in infants and children by S. Spector *et al.*, (*Pediatrics* **22,** *515 (1958)*).

Thirty-three convalescent patients whose fat absorption was assumed to be normal received 1 ml. per kg. of body weight of corn oil labeled with I^{131} emulsified in water and orange juice. Blood samples were obtained at 2, 4, 6, 8, 12, and 24 hours and the urine and stools were collected for 48 hours. Preliminary studies had shown that less than 8 per cent of the I^{131} was split off from the corn oil during simulated digestion by dog intestinal contents.

Infants under one year of age absorbed an average of 78 per cent of the ingested corn oil. Those between one and two years of age absorbed an average of 90 per cent, and the children over two years of age absorbed an average of 97 per cent. The postabsorptive concentrations of labeled fat in the blood were expressed as per cent of the administered dose calculated from a theoretical total blood volume. In all three age

groups the shape of the ascending curves and the peak levels reached at four hours were similar. In the group under one year of age the concentration fell rapidly after four hours. In those over two years it was sustained for at least eight hours. Despite the significant differences in fat absorption, the blood curves tended to overlap to a large extent.

Thirteen patients with cystic fibrosis of the pancreas had an average absorption of labeled fat of 44 per cent, ranging from 23 to 66 per cent. In nine of 11 retested using pancreatin (triple strength U.S.P.) the absorption of labeled fat increased to an average of 83 per cent. The patterns of the concentration of labeled fat in the blood following the test meal were flat in these patients but approached normal following the use of pancreatin.

The results in this study show poorer fat absorption during the first year of life than had been reported by investigators using classical balance studies. These authors point out that they used convalescent patients who were often at the lower percentiles for weight. They also suggested that the I^{131} labeled fat was predominately saturated and consisted largely of 18-carbon fatty acids. It was apparent that the serum concentrations of the tagged fat bore little relationship to the quantitative measures of fat absorption.

Using essentially the same technique K. Reemtsma, P. A. di Sant'-Agnese, J. R. Malm, and H. G. Barker (*Pediatrics* **22,** *525* (*1958*)) investigated the serum and urinary patterns following the oral administration of both I^{131}-labeled neutral fat and free fatty acid in eight patients aged six to 18 years with cystic fibrosis of the pancreas and deficient tryptic activity. The serum content of labeled fat was consistently low, the peak being under one per cent of the administered dose compared with a normal of about six per cent. Following fatty acid ingestion the mean serum content at the peak was 2.6 per cent among the patients as compared with a control level of 7 per cent. The urinary recovery rate of I^{131} was greatly reduced following the administration of neutral fat when compared with the control subjects. Following the administration of the free fatty acid, however, the differences between groups were quite small.

These observations were interpreted as evidence for a nonpancreatic factor in patients with cystic fibrosis of the pancreas, since the absorption of I^{131} labeled fatty acid is normal in depancreatized dogs and in adults with other types of pancreatic disease. The depression of the serum concentrations of labeled fatty acids and the essentially normal cumulative absorption as estimated by the urinary excretion of the label were interpreted as a reduced rate of absorption with little diminution of the total amount absorbed.

The application of I^{131} labeled fats to the problems of fat absorption in cystic fibrosis of the pancreas has resulted in a diagnostic tool that will probably have widespread use. The double tolerance test using both neutral fat and fatty acids has already suggested that there are factors in this disease which cannot be completely explained on the basis of reduced intestinal lipase activity.

HYPOCHROMIC ANEMIA OF INFANCY

Iron deficiency with hypochromic anemia remains a common nutritional deficiency disease in the United States. The majority of the patients are found among infants. Most of the recent contributions to the subject have concerned the mechanism of iron deficiency in sucklings (*Nutrition Reviews* **11,** *163* (*1953*)), the fate of transplacentally acquired iron using its radioactive isotope (*Ibid.* **14,** *337* (*1956*)), and studies on the

absorption of food iron using radioiron-tagged foods (*Ibid.* **16,** *170* (*1958*)). The appearance of two papers dealing with the multiple factors involved in the clinical entity of hypochromic anemia in infancy has re-emphasized our somewhat limited understanding of the etiology of this deficiency state.

C. W. Woodruff (*J. Am. Med. Assn.* **167,** *715* (*1958*)) studied 272 cases of hypochromic anemia in infants whose hemoglobin concentration was less than 9 g. per cent. These cases were collected over a three year period and represented approximately one per cent of all the pediatric admissions during this time. The age distribution of the patients extended from three months to 30 months with occasional instances of anemia up to the age of five years. There was a preponderance of male infants. The birth weight distribution of the anemic patients showed them to be considerably smaller at birth than average, with an incidence of 31.1 per cent premature births. Fewer first born infants were found than would be expected, and increased numbers of infants having a birth order of four or five. The more severe cases were almost all found among the premature infants. The incidence of twins in this group was higher than would be expected in all the race, sex, and birth weight groups.

This survey has suggested that the predisposing factors of low birth weight, high birth order, twinning, and the more rapid growth of male infants played a very significant role in the development of severe hypochromic anemia. It was also implied by Woodruff that these predisposing factors were of great importance in the development of iron deficiency since it appeared that the diets consumed by the anemic infants were not greatly different from those consumed by other infants in this same population who did not show such severe degrees of anemia.

J. P. Dawson and J. F. Desforges (*Am. J. Dis. Child.* **96,** *169* (*1958*)) undertook to evaluate the relative importance of inadequate iron storage at birth, excessively rapid growth, and decreased dietary iron intake in a rather similar group of infants. The age incidence showed that the majority of the cases occurred between the ages of 10 and 20 months. There was a predominance of male infants, of prematures, and of twins. The relatively faster growth of the premature infants was beautifully demonstrated by expressing weight as multiples of birth weight at various ages.

The serum iron values of the anemic infants were all less than 48 micrograms per cent. Six out of 29 mothers of full term infants had serum iron values which were below normal. Similar observations on the mothers of 11 premature infants revealed five with low serum iron concentrations. When the groups were considered together, the average serum iron of the non-pregnant mothers of anemic infants was less than that of normal women who had not been pregnant, or less than that of a group of control mothers from the hospital ward, but these differences for the group were not statistically significant. However it seems certain that, at least in some cases, maternal iron deficiency contributed to the deficiency as well as the rapid growth rate of the infants. Fifty-three of the 77 infants studied had diets which were considered abnormal because of either excessive milk intake or decreased intake of solid foods.

In attempting to classify the role of these three factors in the development of iron deficiency, Dawson and Desforges found that, in the 54 cases where the information appeared adequate, 28 were found to have two or more factors contributing to the anemia (that is, a combination of low birth weight and deficient diet, or rapid growth and evidence of maternal iron depletion, and so forth). In the 18 cases, or 33 per cent, in which only one factor appeared to be responsible, this was most frequently a diet considered to be deficient. Although a low

maternal iron store was never the only abnormality found, it did appear that this factor was of considerable importance in the development of iron deficiency in the small, rapidly growing infants who were not consuming adequate diets.

While there is no question that the intake of the amounts of iron generally considered to be adequate for infants will prevent the development of hypochromic anemia, it becomes increasingly obvious that many other predisposing factors can be found among infants ingesting less than adequate amounts of iron. These factors are maternal iron deficiency, low birth weight, and rapid growth. The high incidence of prematurity in both of these studies would suggest further that the prevention of hypochromic anemia in these small infants has not been universally successful.

VITAMIN E AND CYSTIC FIBROSIS OF THE PANCREAS

Steatorrhea has been one of the major clinical manifestations of fibrocystic disease of the pancreas. Our knowledge of the specific defects in fat absorption in these patients is increasing. The findings of low serum tocopherol levels, an increase in susceptibility to peroxide hemolysis of the red cells, and creatinuria have recently been reported in this disease (*Nutrition Reviews* **15,** *135* (*1957*)). These results have been attributed to the effects of a long standing defect in fat absorption involving vitamin E. The metabolic effects were abolished by suitable tocopherol therapy. Changes in the striated muscle similar to those observed in experimental animals with avitaminosis E were found in one patient.

Evidence that the long term effects of relatively poor vitamin E nutrition in patients with cystic fibrosis of the pancreas may result in ceroid pigment deposition has been obtained by W. A. Blanc, J. D. Reid, and D. H. Andersen (*Pediatrics* **22,** *494* (*1958*)). In reviewing their autopsy experience of 151 cases, 82 were selected in which sections of the smooth muscle fibers of the esophagus, ileum, and appendix were available for correlation of the amount of ceroid pigment with age. Ceroid pigment is a finely granular, refractile substance located at the poles of the nuclei in smooth muscle fibers and was identified and graded by examination of routine sections. Each case was then scored by addition of the grades on the three sections, with the final scores varying from zero to six.

In 35 patients under one year of age no ceroid pigment was seen. One of the five patients between one and two years of age showed some ceroid deposition and this only in the appendix. Two of the four patients in their third year showed this abnormal pigment. After the age of three years, however, ceroid pigment was present in 37 of the 38 individuals examined. The four patients over the age of ten years received a score of five or six. There was no sex predominance. In seven patients having only a partial deficiency of proteolytic enzymes when initially observed, there appeared to be less ceroid pigmentation. There was also a suggestion that there was some correlation between early diagnosis with adequate treatment and low ceroid scores.

In 18 instances of biliary atresia, a disease in which fat absorption is greatly diminished, there appears to be a similar age distribution of ceroid scores except that an earlier onset was usually present. None of four children with celiac disease, and three out of 13 with portal cirrhosis, showed ceroid deposition in the smooth muscle of the intestinal tract.

The control material was divided into two groups. None of the 50 appendices removed from healthy children of ten years or older showed any ceroid deposit. None of the 392

patients not having pancreatic deficiency, cirrhosis of the liver, or biliary atresia showed any ceroid deposition. Thus it would seem that ceroid deposition in the smooth muscle of the gastrointestinal tract was limited to those diseases which are characterized by a defect in fat absorption present for several years.

A search was made for anatomic evidence of tocopherol deficiency in the striated muscle in these cases. There were pathologic changes in only three and none showed any ceroid pigment deposition.

The deposition of ceroid pigment in most organs of laboratory animals maintained on diets deficient in vitamin E has been attributed to the antioxidant effects of the vitamin on unsaturated fatty acids (*Nutrition Reviews* **13,** *250 (1955)*). This deposition is prevented by the administration of tocopherol, but the amount of pigment already deposited is not affected by subsequent treatment. The pigment of experimental avitaminosis E and the ceroid observed in human cases appear to be identical histochemically and morphologically. Since this was entirely a retrospective study no observations concerning serum tocopherol levels or *in vitro* peroxide hemolysis of the red cells were available.

Blanc, Reid, and Andersen suggest that the lesions in the gastrointestinal tract of patients with cystic fibrosis of the pancreas represent the cumulative effect of avitaminosis E over the lifetime of these individuals. Since the long standing defect in fat absorption found in these patients actually does produce a measurable degree of tocopherol desaturation of the serum and presumably of other tissues as well, such a hypothesis seems warranted. Long term study of children with this disease and without tocopherol supplementation must be carried out before any final conclusions concerning this relationship can be made.

INCORPORATION OF DIETARY CALCIUM INTO GROWING BONE

Although several tracer studies have been carried out with objectives of measuring rates of incorporation of radio-labeled dietary calcium into bone (*Nutrition Reviews* **15,** *92, 198 (1958)*), these have been complicated by the reconstruction and other exchange processes of the particular bones studied. S. Tsurufuji (*J. Biochem.* **45,** *163 (1958)*) has sought to bypass these complexities by using, as the test object for incorporation, the continuously growing incisor teeth of rats. In these structures, reconstruction is at a minimum and the rate of growth is estimated to be approximately that which would take place in other bones if the erosion processes are left out of consideration.

Growing albino rats (90 to 110 days old) on a calcium-deficient diet were given 70 to 90 mg. of calcium daily, as the carbonate, by intubation. In the experimental group, the calcium carbonate was labeled with Ca^{45}. It was found, by weight checks, that calcium administered in this manner was nutritionally satisfactory. The rats were sacrificed 24, 48, and 72 hours after ingestion of the labeled calcium carbonate.

The specific activity of the calcium available for incorporation into the incisor teeth was considered to be equal to that of the calcium in the serum. In the present experiments, the specific activity of the serum calcium was found to have remained constant during the period 48 hours to 72 hours after administration of labeled calcium. In such a case, the total amount of deposited calcium can be computed simply. It was found that 0.59 mg. of calcium was deposited into one lower incisor tooth during a 24 hour period. This value compares very well with an estimate of 0.62 mg. of calcium per day based on the technique of repeated injections of radio-labeled calcium into the circulation (Y. Ito, S. Tsurufuji, Y. Hira-

matsu and M. Chiba, *J. Biochem.* **43,** *701 (1956)*).

The actual amount of labeled dietary calcium taken into the incisor tooth is small compared to that taken from other sources. Only 0.188 mg. of calcium from the diet appeared in the tooth, whereas the total calcium taken up by the tooth was approximately 0.6 mg. as estimated above. In a previous communication by the author (Y. Ito, S. Tsurufuji and E. Muria, *J. Biochem.* **44,** *195 (1957)*), it was reported that even 12 days after the ingestion of labeled calcium, the amount of the labeled dietary calcium found in the lower incisor was only 0.433 mg., as compared with 0.6 mg. of total calcium deposited daily by new tissue formation. On the other hand, the bone tissues other than teeth took up more labeled dietary calcium than that expected from calcium retention (bone growth) (H. E. Harrison and H. C. Harrison *J. Biol. Chem.* **185,** *857 (1950)*).

The author feels that these observations are consistent with the idea that calcium absorbed from the gastrointestinal tract is deposited not only through bone growth but also by physico-chemical exchange and biological reconstruction processes not directly related to bone growth. In a previous experiment (Y. Ito *et al., J. Biochem.* **44,** *195 (1957)*), it was found that when 155 mg. of labeled Ca was ingested by rats over a three-day period, 109.5 mg. of calcium were retained. However, this amount of calcium had not all been used for bone growth during the three days because the rate of incorporation of labeled dietary calcium into bone materials through the formation of new bone mineral must have been as slow as the incorporation of labeled dietary calcium in the incisors. Twenty-two per cent of the labeled dietary calcium deposited into the incisor was from new dental minerals. Thus, 15.6 per cent of the labeled dietary calcium found in the animal's body could be accounted for by new bone growth ($109.5 \times 0.22/155 = 15.6$ per cent). Physico-chemical exchange and biological reconstruction processes were responsible for the deposition of another 84.4 per cent of labeled calcium into the skeletons of the rats.

CORTISONE AND PARATHYROID EXTRACT EFFECTS IN RATS

Both cortisone and parathyroid extract cause changes in teeth and bones, alter the blood level of calcium, and affect the kidneys. However, the effects of each are different. Z. Laron, J. Muhlethaler and R. Klein (*Arch. Path.* **65,** *125 (1958)*) injected cortisone and parathyroid extract either separately or combined in the same rats and studied the histological and chemical effects over a six day period. All rats received a constant, suboptimal amount of ground chow to ensure complete diet consumption.

The control rats maintained their weight around 150 g. The rats getting 200 units of parathyroid extract (hereinafter called P.T.E.) lost about 5 per cent of their weight while groups getting 10 mg. of the cortisone alone or in combination with P.T.E. lost about 9 per cent of their body weight. These dosages are extreme when one considers the normal circulating levels of the hormones or even the experimental doses which result in weight loss.

Serum calcium was higher in the P.T.E. group and lower in the cortisone group than in the control animals. The group getting the combined treatment had a calcium value approaching that of the control group. The phosphorus level was not appreciably affected by either P.T.E. or cortisone. Serum alkaline phosphatase was lowered by cortisone only in some rats. The P.T.E. and cortisone combined resulted in lowered serum alkaline phosphatase but the difference was not significantly lowered in cortisone treated animals.

The epiphyseal cartilage plate was widened and thickened in P.T.E. treated animals and contained clear, vacuolated cells, contrasting with the small, eosinophilic cells in the control animals. Although osteoclasts were present, resorption was poor resulting in broad, cartilaginous cores and dense primary spongiosa. Cortisone caused thinning of the epiphyseal cartilage. Resorption and osteogenesis were repressed and the secondary spongiosa was porotic. The rats receiving both cortisone and P.T.E. showed changes similar to, but less pronounced than, the changes in animals given P.T.E. alone.

The teeth of animals getting P.T.E. showed diminished calcification of the dentin. Cortisone, however, did not affect the dentin but caused a widening of the predentin. In the group getting both hormones, little change in tooth structure was observed.

Kidneys of rats treated with P.T.E. showed calcification of the proximal tubules and calcified casts in the distal tubules. Kidneys appeared normal in rats injected with cortisone or both hormones.

There are many questions remaining to be answered about the interactions of P.T.E. and cortisone. Dietary factors that may influence the appetite should be studied. Varying dosages of each hormone might produce different effects. Also a longer experimental period should yield more information on the dental and osseous changes. Nevertheless, this study indicates a protective effect of cortisone on several of the deleterious effects of P.T.E. administration.

In summary: Rats injected with large doses of cortisone and/or parathyroid extract were studied in acute experiments lasting six days. The P.T.E. increased the serum calcium level but cortisone decreased it. Citric acid was not affected by P.T.E. but was diminished by cortisone. The effects of parathyroid hormone in bones were prevented by cortisone as was the calcification of kidney tubules. The mechanism of the effect was not evident.

NITROGEN ASSIMILATION BY ALGAE

In algae such as *Chlorella ellipsoidea*, nitrogenous substances, such as ammonia, urea, nitrate, arginine, ornithine and citrulline are readily assimilated. Presumably, this occurs at the expense of energy liberated by respiration which is always found to be markedly increased upon addition of these nitrogen sources (A. Hattori, *J. Biochem.* **44,** *253 (1957)*). Hattori (*Ibid* **45,** *57 (1958)*) has recently investigated in greater detail the process of assimilation by this organism of ammonia and urea. Changes taking place were followed with reference to the nitrogenous constituents, amides and amino acids, present in the soluble and non-soluble nitrogen fraction of the cell.

In an attempt to promote increased uptake of ammonia and urea and to facilitate detection of assimilated nitrogen the culture of *Chlorella ellipsoidea* was pre-starved with respect to nitrogen. Washed cells were suspended in a nitrogen-free salt medium (pH 5.5–5.6) and solutions of urea, ammonium sulfate or l-arginine were added. Aliquots of the suspension were removed immediately after the additions of nitrogenous materials and at various time intervals thereafter.

When ammonium sulfate was added to nitrogen-starved cells of *Chlorella*, ammonia was rapidly assimilated and organic nitrogenous compounds were formed. After three hours, about 60 per cent of the assimilated ammonia was present in the cells in soluble form; the remainder had been converted to insoluble compounds. During the initial period the increase in soluble nitrogen was due largely to amide, especially glutamine. Thereafter, a steady increase in free amino nitrogen was also observed. Among the amino acids investigated, arginine showed the largest increase. Ornithine, total acidic amino acids, glutamic and aspartic acids

were also slightly increased. There was no net increase of citrulline or of urea.

During the first four hours following the addition of urea to the cellular suspension, almost all of the nitrogen of the assimilated urea was present in soluble form. Thereafter, it was gradually incorporated into insoluble compounds. As there was no net accumulation of urea, it was assumed that urea was taken up at a rate which was somehow dependent upon its conversion into other nitrogenous compounds. The amount of amide nitrogen was increased slightly and gradually in contrast with the rapid increase observed following assimilation of ammonia. However, there occurred a larger and more rapid increase of arginine than was the case when amonia was assimilated. Other amino acids were also increased, but there was no change in the content of total acidic amino acid.

When arginine itself was given to the cells, a temporary accumulation within the cells occurred followed by a conversion to other compounds such as asparagine.

The importance of amide as intermediates in the assimilation of ammonia was earlier demonstrated by P. J. Syrett (*Ann. Bot., N.S.* **17,** *21 (1953)*) who used, as a test organism, *Chlorella vulgaris*. E. W. Yemm (Symposium on *Recent Developments in Cell Physiology*, Academic Press, Inc., New York, Edited by J. A. Kitching (1954)) has also presented evidence for the primary synthesis of glutamine from ammonia in barley roots. This was followed by secondary conversion into asparagine and various amino acids. In the case of urea assimilation, however, it appears from the present investigation that the primary intermediate may be arginine.

The possibility that urea is assimilated by conversion to ammonia was excluded by two other pieces of evidence: (1) there was no trace of urease activity detectable in *Chlorella* cells and (2) no trace of ammonia could be detected during the process of urea assimilation. Although the author feels that it is probable that Krebs' ornithine cycle operates in the process of urea assimilation, the only evidence for it was the increase in ornithine during incubation with ammonia and urea as well as arginine. The addition of ornithine to the reaction mixture caused no acceleration or decrease of urea assimilation.

Although ammonia and urea are not utilized to any appreciable degree by mammalian cells, studies of their utilization in single celled organisms have practical nutritional aspects (1) both in terms of better understanding of metabolic mechanisms and (2) with respect to a possible economical method of producing protein for animal and human consumption.

RATE OF OXIDATION OF LIPIDS

There has been a marked upsurge of research activity in the field of lipid metabolism during the past two decades. The results of this work show that hydrolysis of triglycerides does not have to occur prior to absorption (*Nutrition Reviews* **15,** *307 (1957)*). When the intact triglycerides are absorbed, they enter the lymph. When hydrolysis occurs, the longer chain fatty acids are absorbed via the lymphatic system while the shorter chain acids are absorbed via the portal vein. The 12-carbon fatty acids are absorbed partly by each of these routes (*Ibid.* **14,** *308 (1956)*). While the fats are being absorbed into the thoracic duct, changes in the length of the fatty acids and in the degree of unsaturation may occur, as shown by the work of F. H. Mattson (*Ibid.* **14,** *309 (1956)*).

Once the triglycerides reach the lymphatic system, they are present primarily as chylomicrons. The mechanism whereby chylomicrons are formed is unknown. Analysis shows that the chylomicrons obtained from thoracic duct lymph of dogs contains in per cent: cholesterol esters 1.5

to 2.0; free cholesterol 0.7 to 1.0; phospholipids 8.2 to 8.9; triglycerides 86.3 to 86.5; unesterified fatty acids 0.2; protein 2.2 to 2.5 (R. J. Havel and D. S. Fredrickson, *J. Clin. Invest.* **35,** *1025 (1956)*). The above values are similar to those for chylomicrons from human beings (Havel and Fredrickson *loc. cit.*). The phospholipids presumably form the outer layer of the chylomicrons and play an important role in stabilizing the emulsion of lipids in the plasma. Evidence for this postulate comes from the observation that the enzyme lecithinase breaks the emulsion of chylomicrons in lipemic rat blood (J. J. Elkes and A. C. Frazer, *J. Physiol.* **102,** *24P (1943)*).

Since triglycerides and phospholipids constitute the major fraction of chylomicrons, it was logical that attention should be given to the fate of these components. E. L. McCandless and D. B. Zilversmit (*Am. J. Physiol.* **193,** *294 (1958)*) have studied the rate of disappearance of these compounds from both artificial lipid emulsions and chylomicrons when they were injected into the blood of dogs.

The artificial emulsions contained 10 per cent coconut oil, 1 per cent soybean lecithin and 5 per cent glucose or 10 per cent glycerol suspended in water. When the above suspension was prepared with a tracer amount of I^{131}-triolein, the triglyceride fraction disappeared very rapidly from the plasma of dogs that had received the emulsion by injection. Each dog received 50 ml. of the emulsion while it was in the post-absorptive state. The half-life for the labeled triolein in ten unanesthetized dogs ranged from one to five minutes (average, two and nine-tenths). When the emulsion was prepared with P^{32}-labeled lecithin, the half-life of the phospholipids in eight dogs ranged from 180 to 390 minutes (average, 278).

There is a possibility that the phospholipid in the artificial emulsion was present in excess of that required for the stabilization of the triglyceride, and this might have been one factor for the prolonged disappearance time of the phospholipid. To investigate this problem and to determine whether the rapid rate of removal of the triglycerides also occurred with natural fat emulsions, McCandless and Zilversmit (*loc. cit.*) secured lymph from dogs with thoracic duct fistulas. To label the chylomicron triglycerides, the dogs with the thoracic duct fistulas were given a dose of I^{131}-labeled triolein by stomach tube. The lymph collection from the donor dogs occurred at the rate of 10 ml. per hour. Radioactivity appeared in the lymph within half an hour after feeding the labeled fat, with peak values appearing in the first three to four hours; however, collections as late as nine hours after feeding contained considerable amounts of radioactivity. When 20 to 40 ml. of the lymph was injected into normal unanesthetized dogs, the half-life for the triglyceride fraction ranged from five to 15 minutes, with the average nine and two-tenths minutes.

Using essentially the same technique, McCandless and Zilversmit (*Am. J. Physiol.* **191,** *174 (1957)*) found that P^{32}-labeled lymph phospholipids had half-time disappearance curves in dogs ranging from 210 to 270 minutes. They intended to determine simultaneously the disappearance rates of triglycerides and phospholipids, both labeled with I^{131}, but were unable to do so because of analytical difficulties in quantitatively separating the two lipid components. It was for this reason that they had to use the data obtained with the P^{32}-labeled phospholipid.

Since there was still a possibility that an excess of phospholipids beyond that actually present as a part of the chylomicrons might be present in the lymph, McCandless and Zilversmit (*Am. J. Physiol.* **193,** *294 (1958)*) separated the chylomicrons from the lymph by ultracentrifugation. When the chylomicrons containing I^{131}-labeled triglycerides were resuspended in saline and injected into dogs, the half-time of disappearance curves the same as that for the whole lymph (*i.e.*, nine minutes). When similar experiments were carried out with chylomicrons con-

taining P^{32}-labeled phospholipids, the half-time disappearance curve was the same as when the whole lymph was given (*i.e.*, 220 minutes). Finally, they measured in the same dogs the rate of disappearance of triglycerides (as determined by chemical analysis of the total fatty acid fraction) and the P^{32}-labeled phospholipids in the chylomicrons. To correct for the triglycerides originally present in the blood, the level of these compounds in the post-injection sample of blood was subtracted from the values in the samples taken subsequent to the chylomicron injection. Here again, the half-times of triglyceride disappearance curves ranged from 5 to 15 minutes (average, $8\frac{6}{10}$ minutes). The half-time for phospholipid disappearance curves in the same dogs as determined by the loss of radioactivity from the blood ranged from 110 to 360 minutes (average, 220 minutes).

Actually, the observations of McCandless Zilversmit confirm those of D. S. Goldman and co-workers (*J. Biol. Chem.* **184,** *719 (1950)*) who found that when a triglyceride emulsion containing C^{14}-labeled palmitic acid was injected intravenously into normal dogs, only from 2 to 3 per cent of the injected C^{14} could be detected in the plasma one hour after injection. The calculation of the half-life of the labeled palmitate was 39 minutes in one dog and 42 minutes in the other dog. These workers injected the labeled acid as tripalmitin in an olive oil suspension stabilized with glycerol monostearate.

The rapid disappearance rate of the triglycerides from the blood may represent the speed with which they are utilized by the body. Some evidence on this point was presented by Goldman *et al.*, *loc. cit.* who found that fairly large amounts of C^{14}-labeled carbon dioxide was exhaled by the dogs injected with triglyceride containing C^{14}-labeled palmitic acid. The slow disappearance of the phospholipid fraction suggests that it follows a metabolic pathway that is different from that of the triglycerides and that phospholipids remain in an intact state in the blood for fairly long periods of time. These observations emphasize the fact that although phospholipids may form an integral part of the chylomicron, they are metabolized at a much slower rate than are the triglycerides. McCandless and Zilversmit (*Am. J. Physiol.* **193,** *294 (1958)*) suggest that the phospholipid component of the chylomicrons forms a reservoir within the plasma and lymph which is used over and over again in forming an emulsifying surface around the triglyceride particles.

DIETARY FACTORS INFLUENCING FLUORIDE RETENTION

The influence of dietary fat on the retention of fluoride in the skeleton and soft tissues has recently been investigated in two laboratories.

At the University of Wisconsin, it was observed that fluoride toxicity in rats was enhanced when the cottonseed oil, in a ration containing 0.1 per cent of sodium fluoride, was increased from 5 per cent to 15 or 20 per cent. Growth retardation and increased deposition of fluoride in bone (femoral) were evidence of increased toxicity with the higher fat rations (R. F. Miller and P. H. Phillips, *J. Nutrition* **56,** *447 (1955)*).

At the University of Indiana, it was noted that dietary fats enhanced the retention of fluoride in the soft tissues when rats ingested 2 mg. of fluoride daily (W. Buttner and J. C. Muhler, *Ibid.*, **63,** *263 (1957)*). Evidence has also been reported from the Indiana laboratory that the presence of vitamin C in the diet enhances the retention of fluoride in both the skeleton and soft tissues of the guinea pig (Muhler, *J. Am. Dent. Assn.* **56,** *335 (1958)*).

A recent report from the Indiana laboratory is concerned with additional studies dealing with the retention of fluoride as

influenced by dietary fat intake in the rat (Buttner and Muhler, *J. Nutrition* **65,** *259 (1958)*).

Five series of trials, involving 150 male weanling rats, were conducted. Series I was divided into three groups, all of which received the basal ration containing 5 per cent of cottonseed oil. Group one received no added fluoride; group two received 30 micrograms per ml. (30 ppm) of sodium fluoride in the drinking water; and group three received 2 mg. of sodium fluoride per animal daily by stomach tube. The same group treatments were repeated in trial series II through V with the fat level and source in the basal ration as follows: Series II, 20 per cent of cottonseed oil; series III, 20 per cent of corn oil; series IV, 20 per cent of hydrogenated vegetable oil; and series V, 20 per cent of lard. The drinking water (fluoride-low) for the animals in groups one and three contained 0.05 micrograms per ml. of fluoride.

The basal ration was of the purified type and contained 30 per cent of vitamin-test casein, 4 per cent of salts, 1 per cent of vitamin mix, and sucrose to equal 100 per cent.

After an experimental period of ten weeks, all animals were sacrificed, and the fluoride content was determined in the femora, whole carcass, liver, kidney and heart.

Neither the fluoride levels nor the fat levels tested had any influence on body weight. In this connection it should be pointed out that the maximum fluoride level used here was only about one-fifth the level used by Miller and Phillips (*loc. cit.*) when they demonstrated growth-depression as an expression of enhanced toxicity for diets containing 20 per cent of cottonseed oil.

In the control animals (no added fluoride), the Indiana workers found a tendency toward increased storage of fluoride in the femur when the dietary cottonseed oil was increased to 20 per cent at the expense of dietary sucrose on an equal weight basis (125 ppm *vs.* 98 ppm). This difference became highly significant when the animals received 30 ppm of fluoride in the drinking water (2610 ppm *vs.* 2180 ppm). A similar, although not significant, increase in femoral fluoride occurred when the rats received 2 mg. of fluoride daily with the 20 per cent cottonseed oil ration (6440 ppm *vs.* 5620 ppm).

The ingestion of corn oil, hydrogenated vegetable oil, or lard resulted in significantly higher femoral fluoride retention values than the ingestion of the 5 per cent cottonseed oil basal ration when the fluoride was administered by stomach tube. When the fluoride was consumed in the drinking water, however, the concentration of femoral fluoride was lower with these fats than was observed with the basal ration. Since the 2 mg. per rat per day intake via stomach tube exceeded the apparent fluoride intake from drinking water containing 30 micrograms per ml. by a factor of about 2.5, it is not possible to make a direct comparison as to the influence of mode of administration on fluoride storage. The results make it clear, however, that the fat source as well as the total fluoride intake had an influence on bone storage of fluoride.

The results of whole carcass analyses were not consistently in harmony with the results obtained with femora, which emphasizes that a single bone may not serve as a representative tissue when fluoride retention is studied. For example, on the 20 per cent corn oil diet, the total fluoride stored in the carcass from 30 ppm fluoride in the drinking water, and the concentration of fluoride in the carcass, exceeded those observed for the basal ration, whereas the femoral retention data already mentioned indicated the reverse for this particular dietary fat.

The results of soft tissue analyses indicated that all of the fat sources tested (20 per cent level) resulted in a greater retention of fluoride than was observed with the basal ration containing 5 per cent of cottonseed oil, the one exception being heart tissue. Combining the data for the two modes of

fluoride administration, liver tissue, for example, contained about 1.5 ppm of fluoride on the higher fat rations, contrasted with a concentration of about one-half this amount on the basal ration. In the case of heart tissue, it is of further interest that fluoride in drinking water or via stomach tube did not appear to enhance fluoride retention in this organ over that observed in the animals not receiving added fluoride.

Relative to soft tissue retention of fluoride, the Wisconsin workers found soft tissues in dairy cows (heart, liver, kidney, pancreas, thyroid and adrenal) to contain from 2 to 3 ppm of fluoride when the cows were fed a basal ration containing 3 to 5 ppm of fluoride. The addition of 50 ppm of dietary fluoride increased soft tissue concentration of fluorine by a factor of 2 to 3. They concluded that the narrow margin in concentration between normal and fluorosed tissue makes the use of soft tissue analysis an unreliable one as a criterion of fluorine toxicosis (J. W. Suttie, P. H. Phillips and R. F. Miller, Jr., *J. Nutrition* **65,** *293 (1958)*).

These studies are of considerable interest. It is obvious that additional studies are needed to determine the significance of dietary fat as a factor in regulating the concentration of fluoride, particularly in soft tissues. The possible physiological role, if any, of fluoride in such tissues, is, of course, also not understood at present.

BIOTIN IN PROTEIN SYNTHESIS

The specific participation of biotin in a number of biochemical processes is well established. Biotin is required in bacteria and yeasts for synthesis of aspartic and glutamic acids (J. L. Stokes, A. Larson and M. Gunnes, *J. Bacteriol.* **54,** *219 (1947)*), and biotin deficiency (in ducklings) results in interference with pyruvate and succinate oxidation in the citric acid cycle. It has been shown that biotin takes part in the fixation of carbonic acid in cells of higher animals as well as in microorganisms (H. A. Lardy and J. Adler, *J. Biol. Chem.* **219,** *933 (1956)*). The synthesis of the higher unsaturated fatty acids in microorganisms is also interfered with by biotin deficiency (H. C. Lichstein, *Vitamins and Hormones* **9,** *27 (1951)*). However, in spite of these many individual observations, biotin remains one of the few B-group vitamins about which no unanimous opinion exists regarding its exact role in metabolic processes.

Since the scattered data allow no conclusion regarding the connection between biotin and protein synthesis, A. A. Poznansksys (*Biochemistry* **22,** *626 (1957)*) has undertaken a study of the effect of biotin deficiency on serum albumin synthesis by liver slices and on amylase synthesis by slices of pancreas.

Biotin avitaminosis was induced in chicks (white Leghorns, four to seven days old) by feeding a synthetic diet containing dried eggwhite. It is well known that avidin, contained in the white, forms a biologically inactive complex with biotin making it unavailable to the animal. Weekly doses were given of 7000 I.U. of vitamin A and 400 I.U. of vitamin D. Each chick received a daily adequate supplement of the following vitamins: thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, choline, inositol, vicasol and folacin. Control chicks on the same synthetic diet received 10 micrograms of biotin subcutaneously twice a week. Symptoms of biotin deficiency appeared in all experimental animals within three to four weeks. Control chicks developed well and gained weight rapidly.

In pancreas slices from vitamin-deficient chicks, amylase formation took place at a very low rate (not exceeding 15 units per mg. tissue) whereas amylase activity in pancreatic slices from normal animals increased

during incubation by 95 to 140 units per mg. Restoration of synthetic activity could be achieved by intraperitoneal injection of 100 micrograms of biotin 24 hours prior to killing.

Synthesis of proteins such as amylase is an endothermal process which is coupled with the cleavage of high-energy bonds of ATP. This process is impaired under conditions where cell respiration and oxidative phosphorylation are depressed. With this fact in mind, Poznansksys noted the effect of supplementing with some of the tricarboxylic acid cycle intermediates a medium in which pancreas slices were maintained. Pyruvate was found to have no effect on amylase formation in pancreatic tissue of the vitamin deficient chicks. Since, during biotin avitaminosis, pyruvate transformation into oxaloacetate (and its subsequent oxidation through the cycle) is inhibited, this result was not unexpected.

In contrast to the effect of pyruvate, supplementation of the medium with α-ketoglutarate increased amylase formation in the slices to a level nearly equivalent to the rate obtained in slices from normal chicks. Supplementation with fumarate resulted in about half the synthesis observed in the case of α-ketoglutarate. The author is of the opinion that this fact further confirms the view that biotin is not only essential in the synthesis of 4-carbon dicarboxylic acids in the respiratory cycle, but is also concerned with their further transformation into α-ketoglutarate.

These conclusions were further supported by a series of experiments in which specific inhibitors of the tricarboxylic cycle were employed in normal chicks. The addition of mesotartarate (which inhibits pyruvate transformation to oxaloacetate) and fluoroacetate (which inhibits the formation of α-ketoglutarate by the respiratory cycle) nearly completely abolished amylase formation. The effect was reversed by addition of α-ketoglutarate.

In liver slices from biotin-deficient chicks albumin synthesis either failed to take place at all or did not exceed 5 per cent of the normal. A single intraperitoneal injection of 100 micrograms of biotin into vitamin-deficient chicks 24 hours prior to the experiment resulted in a normal production of serum albumin.

When attempts were made to restore albumin synthesizing ability to vitamin-deficient liver slices by the addition of α-ketoglutarate, not only did this substance fail in this respect but also it completely abolished albumin synthesis in liver slices from normal chicks! Neither glutamine nor asparagine produced such inhibition in slices from normal chicks, and glutamine did increase the production of albumin from biotin-deficient livers to normal values. Thus the effect expected by the precursors of dicarboxylic amino- and keto-acids, glutamine and asparagine, on albumin synthesis in the liver was analogous to the effect of ketoglutarate and fumarate on the synthesis of amylase by pancreas.

The results are consistent with the idea that biotin does not participate directly in protein synthesis but acts, perhaps as a coenzyme, in the synthesis of dicarboxylic acids of the citric acid cycle which are essential for the processes of oxidative phosphorylation, which, in turn, secure the energy requirements for protein synthesis.

ACTION OF VITAMIN A

Although the mechanism of action of vitamin A in scotopic (dim light) vision is now fairly well known, there has been little indication that this action might be connected to this vitamin's more general function of maintenance of the epithelial tissues (*see Present Knowledge in Nutrition*, 2nd ed., The Nutrition Foundation Inc., New York, 1956, pp. 66–68). As a matter of fact, the general function of vitamin A in pre-

venting atrophy of the epithelial tissues, and consequent susceptibility to bacterial invasion, is poorly understood.

One suggestion has appeared in a study by J. F. Dowling and G. Wald (*Proc. Nat. Acad. Sci.* **44,** *648 (1958)*) which was aimed at outlining the total relationships of night blindness to vitamin A deficiency.

Although the relationship of vitamin A to retinene, rhodopsin and the action of the rods is an obvious and direct one, certain discrepancies have existed. For instance, the time taken to develop night blindness on a vitamin A deficient diet varies greatly with the individual, and the time to cure the deficiency symptom with vitamin A therapy may vary from days to many months. In the first case, the authors considered evidence that the vitamin A stores of the liver, which vary greatly in individuals and with previous dietary habits, might prevent the visual symptoms until the stores were exhausted. In the second case, it seemed plausible that a prolonged deficiency might result in a deterioration of the rods themselves, repair of which would take more time than a simple recombination of retinene with opsin.

In order to study these hypotheses, the authors followed the course of development and cure of vitamin A deficiency in male weanling rats. Measurements were made of the concentration of vitamin A in liver and blood, of rhodopsin in the retina, and of the electroretinographic thresholds and electroretinographs (ERG) over a wide range of light intensities. Opsin was measured by the capacity of the retinas to regenerate rhodopsin when incubated with neo-b (11-cis) retinene. In some cases, retinal histological examinations were also performed.

At the start of the deficient diet, the liver vitamin A content of the rats began to decrease, reaching very low levels at three weeks. Since the rate of decrease was about 2 micrograms per day, as compared with a usual increase of about 45 micrograms per day on a normal diet, it is evident that very young rats had to be used for the experiment. (By way of comparison, depletion of the stores of a well nourished adult human being might take two to ten years). No further symptoms were apparent until the liver supplies were exhausted, when blood levels of vitamin A fell precipitously to zero. At this point, the rhodopsin content of the retinas began to fall, reaching a low level in about three weeks. The outward symptom of this decrease was a rise in the ERG threshold, an indication of the onset of night blindness. At four weeks, before the drop in blood vitamin A, the threshold was unchanged. At five weeks it had increased 14-fold, and at eight weeks, when the animals were succumbing to the deficiency and the rhodopsin content of the retinas was only 16 per cent of normal, the threshold had increased 680-fold. It was found that from four and one half weeks, the log of the ERG threshold, in arbitrary units, rose almost linearly with time on the deficient diet and with the decline of retinal rhodopsin.

The retinal opsin content remained normal through the sixth week, when the retinal rhodopsin was 50 per cent of normal. Thereafter, it began to decline, until at eight weeks it had dropped about 50 per cent and marked deterioration of retinal tissue, especially the outer segments of the rods, appeared. At this point the animals were deteriorating generally, and it was concluded that the opsin was probably only one example of a general destruction of proteins in epithelial structures.

The reversal of these symptoms, with intraperitoneal injection of vitamin A (oral administration gave irregular results) followed the reverse order of their initiation. The ERG thresholds returned to normal in 50 to 60 hours, the time for recovery depending on the extent of damage to the retinal opsin. When the 11-cis isomer was injected, a lag in recovery was apparent, indicating that, although this is the isomer actually used in the retina, only the all-trans is effective in reaching this site. It was evident that the return to normal ERG threshold values presupposed the isomerization of all

trans to the 11-cis isomer (presumably in the eye tissues themselves), the repair or replacement of the degenerated rods and opsin and the combination of opsin and retinene to form rhodopsin. The general recovery of the animals from this stage of deficiency required considerably more time.

These experiments, of considerable interest in themselves, carry implications of even greater import. It appears that the protein opsin, and consequently the outer segments of the rods of which it is an important constituent, are stabilized by combination with retinene and deteriorate rapidly in its absence. The authors suggest that many tissues contain structural proteins which are also stabilized by combination with vitamin A or its derivatives and that these also deteriorate in the deficiency state. If this is true, a logical general explanation is finally available for the function of a previously little understood vitamin.

EVALUATION OF CEREAL CARBOHYDRATES

In 1947 a specialist committee of the Food and Agricultural Organization of the United Nations concluded that "the summary method in which the energy values of 4, 9, 4 are applied to protein, fat and carbohydrate, although in common use, . . . is inaccurate for individual foods and has only limited application to diets". The Committee made a strong recommendation for further research "with special emphasis on the investigation of the components included in 'carbohydrates by difference' as determined by proximate analysis" (*Report of a Committee on Calorie Conversion Factors and Food Composition Tables, F.A.O., Washington, D.C.* (1947)).

The results have now been published of a project carried out in this field by a group of British workers using wheat flours of different extraction and rice flours at varying degrees of refinement (J. R. Fraser, M. Brandon-Bravo and D. C. Holmes, *J. Sci. Fd. Agr.* **7,** *577 (1956)*; J. R. Fraser & D. C. Holmes, *Ibid.* **7,** *589 (1956)*; **8,** *715 (1957)*; **9,** *511 (1958)*). The four carbohydrate components analysed for were "starch", "sugars", "cellulose" and "hemi-cellulose". The components are put in quotation marks here because they each include a range of molecules that may differ from one sample to another. The values obtained are therefore particularly dependent on the details of the analytical procedures used.

Summation of the four carbohydrate components gave, for all the samples tested, an almost exact agreement with the corresponding values for "carbohydrate by difference" (*i.e.*"100—crude protein—ash—fat—moisture"). However, the latter values were obtained with the use of special precautions that are not always adopted in routine laboratories.

At one extreme a sample of 100 per cent extraction wholemeal flour. showed approximately 6 per cent of hemi-celluloses and 4 per cent of cellulose, together with 60 per cent of starch and 2 per cent of sugars. At the other extreme in the wheat flour series, a sample of low extraction rate showed only 2 per cent of hemi-celluloses and 0.3 per cent of cellulose, together with 71 per cent of starch and 1 per cent of sugars. In the series of rice flours there was less variability; in only one sample did the combined non-starch carbohydrates exceed 1 per cent.

As pointed out by J. R. Nicholls and J. R. Fraser (*Proc. Nutr. Soc.* **17,** *43 (1958)*) analytical figures of this kind can serve as a useful control index of the extraction rate of samples of wheat flours. They suggest an index expressed by the sum of the non-starch carbohydrates as a percentage of the "starch + protein" content of the sample. This index has been found to vary from 4 for 72 per cent extraction flour, to 18 for wholemeal flour. It is claimed that this is a more reproducible figure than one based on the crude fiber content of the sample.

However in the present context the main intention in making a more detailed analysis

of the carbohydrates in foods was to allow a more accurate estimate of their energy value in human nutrition. Fraser and Holmes (*loc. cit.*) point out that an exact prediction of energy value from chemical analysis seems unlikely ever to be obtained since digestibility is dependent to some extent on the association between different food components, and on how far the indigestible components protect the normally digestible molecules thus reducing their availability to attack by digestive enzymes.

The cooking of foods hence could increase their energy value by disrupting barriers, without any change in overall chemical composition. In circumstances where there are no reliable figures of energy value for the food in question it is suggested that, as a first approximation, the starch and sugars should be considered as completely available and the other carbohydrates as completely unavailable. This is the principle behind the figures for "available carbohydrate" in official British tables of food composition (*Nutrition Reviews*, **14,** *306* (*1956*)).

W. Bolton (*J. Sci. Fd. Agr.* **8,** *132* (*1957*)) has presented results on the digestibility of carbohydrates by experimental animals that are of interest in this connection. A series of ten foods including the common cereals, cereal by-products, soyabean meal, and peanut meal were analysed for starch, sugars, pentosans, cellulose and lignin; each food was then fed to adult chickens and the digestibility of the components was measured by standardized procedures (Bolton, *J. Agr. Sci.* **46,** *119* (*1955*)).

In all the foods tested the starch and sugars were completely digested and there was negligible digestion of either the cellulose or lignin fractions. However, there was a definite digestion, or at least disappearance, of a fraction of the pentosans from all the foods tested. Among the cereals the highest digestibility value for the pentosans was 38 per cent, obtained with corn; the lowest was 14 per cent, for oats. However, digestible pentosans still provided less than 4 per cent of the total digestible carbohydrates in any of the cereals. In the milling by-products, with much lower starch content, the pentosans provided a greater protein of the total digestible carbohydrate (as high as 13 per cent in the case of bran). In soyabean and peanut meals the pentosans provided about 7 per cent of the digestible carbohydrates.

If the same order of digestibility were to be found with man this would mean some adjustment to the simple measurement of starch and sugars as the only "available carbohydrates". However with the use of more refined foods in human nutrition and their consequent lower pentosan content, the contribution of digestible pentosans would be unlikely to amount to as much as 2 per cent of the total digestible calories provided by a diet. Whether or not such a correction would be of significance in calculating the calorie value of human diets from food tables, with all the further complications of cooking and wastage on the one hand, and the questions of the relative usefulness of "digestible calories" from different molecules for productive purpose on the other, is a matter for speculation.

THE DYNAMICS OF MANGANESE METABOLISM

The essentiality of manganese as an animal nutrient has been demonstrated in careful nutritional studies (*Nutrition Reviews* **15,** *80* (*1957*)). Dietary deficiency has produced a number of symptoms in experimental animals including growth and reproductive failure, abnormal bone growth and abnormalities of the nervous system. Existing information concerning human metabolism of trace metals has been collected by H. A. Schroeder (*Advances in Internal Medicine* **8,** *259* (*1956*)). Spectrographic measurements of human organs indicate that the total pool of body manga-

nese is not much in excess of 10 mg. Little manganese is present in human urine and the feces appear to contain most of the excreted element.

The difficulty inherent in the accurate measurement of manganese in body organs and body fluids has retarded progress in the understanding of the dynamics of manganese metabolism. This methodological handicap has been avoided by Cotzias and his co-workers at the Brookhaven National Laboratory by the application of tracer techniques. L. S. Maynard and G. C. Cotzias (*J. Biol. Chem.* **214,** *489 (1955)*) in their initial studies employed the isotope Mn^{56} which has a half life of 2.59 hours. The disappearance of the isotope from the blood occurred extremely rapidly in both man and the rat. Organ measurements in the rat showed that the liver, pancreas, and kidney were most active in removing the isotope from the blood. The fact that each of these tissues was characterized by a high concentration of mitochondria suggested to the authors that the intracellular distribution of the isotope might determine the observed body distribution. With this question in mind, livers from rats that had received Mn^{56} were homogenized and the organelles fractionated using the familiar methods of Schneider and Hogeboom. As a minimum estimate more than three quarters of the intracellular manganese was found associated with the cellular particles, with mitochondria containing the major share.

Observations in man with Mn^{56} have been greatly extended by D. C. Borg and G. C. Cotzias, (*J. Clin. Invest.* **37,** *1269 (1958)*). The disappearance of the isotope from the blood was examined in great detail. Mathematical analysis of the disappearance curve indicated that it was composed of three components. The initial component with a half-life of only 0.9 minute was attributed to the rapid flux of manganese from the vascular compartment and was of the same order of magnitude as that of most electrolytes. The second component may represent the entrance of manganese into cells. The distribution of manganese in the human body resembles that in the rat, in that a high degree of activity was detected over the liver by external counting. Liver radioactivity reached a maximum in a very few minutes.

A consideration of the blood disappearance curve led the authors to important inferences concerning the labile state of body manganese. On the basis of the relatively stable blood radioactivity level 45 to 60 minutes after the administration of the isotope, it was possible to calculate the specific activity of blood manganese on the basis of available spectrographic measurements of blood manganese concentration. Knowing the amount of injected labeled manganese it was possible to calculate the magnitude of the exchangeable manganese pool. It was found that the size of the calculated exchangeable manganese pool agreed well with the total body manganese as estimated by spectrography. This would indicate that the greater part of the body manganese took part in a very rapid exchange.

The specificity of the pathways of manganese metabolism has been considered in a paper by G. C. Cotzias and J. J. Greenough, (*J. Clin. Invest.* **37,** *1298 (1958)*). Mice were selected for study because of the convenience of whole body radioactivity measurements in this species. The isotopes Mn^{52} (half-life 5.7 days) and Mn^{54} (half-life 310 days) were utilized for these studies because of their longer half-life than that of Mn^{56} previously used. The disappearance of labeled manganese from the mouse's body could be described by two exponential equations, the first with a half-life of 48 to 68 hours and the second with a half-life of 230 to 300 hours. About 20 per cent of the isotope remained in the body after ten days. The importance of gastrointestinal excretion of manganese was established by the direct approach of occluding the anus, thereby blocking further losses of total body manganese. Loss of body manganese was delayed in mice fed a low manganese diet. Intro-

duction of manganese into the diet to the extent of 0.01 per cent and 0.05 per cent led to a progressively rapid disappearance of body manganese.

The predictability of the biologic decay rate of body manganese allowed the effect of the acute administration of carrier manganese and other metals to be observed. Manganese administration in a number of chemical forms was shown to be followed by an acute drop in the total body manganese. Salts of elements related to manganese were similarly injected. These substances included rhenium, magnesium, vanadium, chromium, iron, cobalt, nickel, and copper. Although the chemical properties of a number of these substances overlap manganese, and several members of this group of elements can substitute for manganese in certain isolated biochemical systems, it is remarkable that none of the ions accelerated the loss of manganese from the body or induced significant changes in the distribution of manganese in the separate organs.

These three papers have clearly established several important features of manganese metabolism. First of all, there is an extremely rapid exchange between the manganese of the blood and the cellular pool of body manganese. Secondly, there is evidence of active intracellular concentration of manganese within the mitochondria. Thirdly, the pathway of manganese metabolism has high specificity with little or no interference by ions of related elements. It is as yet impossible to relate these facts of manganese metabolism with the metabolic role of manganese. All the other trace elements of demonstrated essentiality (iron, cobalt, copper, zinc and molybdenum) act in the body tightly bound in porphyrin complexes or bound directly to enzyme systems. The labile physical state of manganese is therefore an important aspect of its metabolism. The intracellular distribution, largely associated with mitochondria, does not correspond with the distribution of enzymes activated by manganese. A specific role of manganese in oxidative phosphorylation in mitochondria has been proposed by O. Lindberg and L. Ernster (*Nature* **173,** *1038 (1954)*) but as yet has not been clearly established. It is hoped that renewed interest in intracellular aspects of manganese action will be stimulated by the important isotopic studies of Cotzias and his associates.

DUODENAL ULCERS IN PANTOTHENIC ACID DEFICIENT RATS

Emphasis in biochemistry has shifted largely from reactions occurring in the whole animal to those that can be studied with tissue preparations, with cell fractions and with highly purified enzymes. The information obtained with these systems is essential for an understanding of many nutritional observations and their importance in providing a fundamental basis for current nutrition research cannot be questioned. Nevertheless, it is important for nutritionists to remember that many of the most intricate details of metabolic reactions are outgrowths of gross observations on the whole animal and that today, as much as ever before, there is a need for experiments on intact animals. Such experiments can serve as starting points for further detailed investigations, and experiments that illustrate how significant many of the well established metabolic reactions are in the economy of the whole animal.

Among the areas in which much exploration remains to be done is the histology and pathology of vitamin deficient animals. A recent contribution to this subject by Zucker and his associates (T. F. Zucker, *Am. J. Clin. Nutrition* **6,** *65 (1958)*) is concerned with the effect of pantothenic acid deficiency on the integrity and functions of the intestines. Their interest in this subject arose from the observation that a deficiency of

pantothenic acid caused the development of severe duodenal ulcers in certain lines of rats.

The ulcers develop in adult rats but usually not until after the eleventh week of the deficiency. They occur characteristically about 2.5 cm. below the pylorus and initially affect the mucosal cells which become necrotic and slough off. Ulcers of long standing may also affect the muscle layers and may eventually perforate. Lesions that are quite severe will heal if the deficient animal is given pantothenate. Scar tissue develops and this is completely covered with regenerated epithelium.

The frequency of occurrence of ulcers, as a result of pantothenic acid deficiency, varies with the strain of rat. Of two non-inbred lines selected for size from a single population, the larger type had an ulcer incidence of 54 per cent, the smaller of only 4 per cent. Other rats obtained from different sources also differed in their susceptibility to ulceration, two strains being quite resistant and one highly susceptible.

In an attempt to find out about the processes involved in the development of these ulcers Zucker and his colleagues examined first the acetylcholine content of the intestinal mucosa. This was done on the assumption that the pantothenic acid deficiency might interfere with acetylation of choline which in turn might affect vascular muscle and thereby the nutriment supply of the mucosa. Resistant rats showed no change in the acetylcholine content of the intestine during the course of the deficiency. Susceptible rats, with a higher initial acetylcholine concentration, showed a decrease, but not below that of the resistant rats and not to a level sufficiently low to prevent the normal response of isolated intestinal segments to treatment with various drugs.

Since acetylcholine synthesis did not appear to be seriously affected, attention was directed toward the possibility that the deficiency exerted an indirect effect through the adrenal cortex. Neither hypophysectomized nor adrenalectomized rats developed ulcers as a result of pantothenic acid deficiency. The removal of only the adrenal medulla did not prevent ulceration.

Since this suggested that adrenal cortical hormone production was altered by pantothenic acid deficiency a series of experiments was carried out in an effort to obtain a measure of adrenal activity. This was done by measuring the effect of pantothenic acid deficiency on thymus weight, which is known to decrease rapidly under the influence of stress or cortical hormone administration. It was evident from this study that thymus weight decreased rapidly when pantothenate was omitted from the diet, suggesting increased adrenal hormone production.

In order to test the validity of this conclusion, adrenalectomized rats were fed either the complete or the pantothenate-deficient diets. Adrenalectomy prevented the decrease in thymus weight previously noted in rats fed the deficient diet. However, when adrenalectomized rats fed either the complete or the deficient diet were injected with identical quantities of adrenal cortical extract, there was a much greater fall in the thymus weights of those consuming the deficient diet. Nevertheless, the thymus weights of adrenalectomized rats injected with 0.2 g. per day of adrenal cortical extract and those of intact rats receiving the same diet, were similar. These observations indicated that pantothenate deficiency did not cause an increase in adrenal cortical hormone production but did cause an increase in the susceptibility of the target organ to adrenal cortical hormones.

In a final experiment, rats of a susceptible line and those of a resistant one were maintained for 13 weeks on a pantothenate-deficient diet. The volume of free acid and the pH of the gastric secretions of both groups and of their respective control animals were measured six hours after gastric ligation. In the susceptible strain pantothenate deficiency caused a rise in the total amount of acid secreted, from 2.3 to 6.5 ml.

of 0.1 N acid, and a decrease in the pH of the gastric contents from 1.73 to 1.35. Adrenalectomy prevented these changes and hydrocortisone administration reversed the effect of adrenalectomy. Pantothenate deficiency was without effect on the ulcer-resistant strain.

To follow the time of development of this sign an insoluble complex of the dye Azure A with an ion exchange resin (Diagnex), from which the dye is released upon exposure to gastric acid, was fed to deficient rats of the susceptible line. The amount of dye excreted in the urine was taken as a measure of gastric secretion. Only after eight weeks on the deficient diet did the susceptible rats show evidence of increased gastric acid secretion. From the eighth to the thirteenth week this increased substantially. The time of this increased gastric acid secretion coincided with the period of ulcer development.

This work provides evidence that there are distinct differences in the response of different non-inbred lines of rats to pantothenic acid deficiency, and that the deficiency signs in older animals may differ from those in young animals. In this case pantothenic acid deficiency caused the development of duodenal ulcers only in adult animals. The time of development of duodenal ulcers coincided with that of increased gastric acid secretion and appeared to be related to hypersensitivity of the target organs to adrenal cortical hormones. This work, apart from its intrinsic value as an extension of our knowledge of the effects of pantothenic acid deficiency, would appear to provide a useful tool for laboratory studies of duodenal ulcers.

AVAILABILITY OF FLUORIDES

Relatively little information has been collected about the availability to man of fluorides from food sources other than water. While natural fluoride-bearing waters and fluoridated water from communal systems provide desirable levels of fluoride that result in major reduction in dental caries for an ever-increasing percentage of our urban populations, many communities are deprived of the benefits of optimal fluoride ingestion because of vocal and often poorly informed or misinformed minorities. Additionally, around 45 per cent of the population of the United States lives in rural areas where communal water supplies do not exist. Hence, there is a continuing need to determine whether vehicles other than water may prove to be suitable.

Many suggestions have been offered: the daily use of pills containing a suitable fluoride compound, or of vitamin preparations to which a fluoride has been added; the fluoridation of table salt by the manufacturer or of milk by the processor; the dispensing of a fluoride solution so that appropriate amounts can be added in the home to water, milk or fruit juice. Each one of these procedures requires the active interest of the consumer and his faithful and persistent adherence to the recommended levels throughout the entire prolonged period while the teeth are being calcified and erupting (from the beginning of the third trimester of pregnancy continuously up to the eruption of the wisdom teeth during early adult life).

On the basis of information from other fields of human welfare, only a relatively small percentage of individuals will avail themselves, on a voluntary basis, of health benefits that have been unequivocally demonstrated by scientific procedures, strongly advocated, and widely disseminated through public information channels. In the case of the ingestion of fluorides from any one of the several available sources for individual use, insufficient information has yet been obtained to determine how effective any

one source (other than public water supplies) may be with respect to the reduction of dental caries. Because of the length of time involved, collection of this information will be arduous, but none the less meritorious.

A. E. Light, F. A. Smith, D. E. Gardner and H. C. Hodge (*J. Am. Dent. Assoc.* **56,** *249 (1958)*) have reported the results of a series of fluoride determinations on teeth from three generations in one family, along with the fluoride content of the water in the community where they resided in their youth. In the case of the grandmother, mother, father, uncle and aunt, fluoride analyses of the drinking water and of the enamel and dentin were consistently low. However, a child in the family had grown up in a low-fluoride community but his mother had consumed, during the last several months of pregnancy, milk containing 1.5 ppm. fluoride as supplied from an appropriate amount of sodium fluoride. In addition, the child had been provided with milk containing 1.5 ppm. fluoride throughout his life.

The fluoride content of the deciduous incisors of this child, at the time that they were exfoliated, contained elevated levels of fluoride far in excess of those of any of his relatives and in amounts consistent with the ingestion of comparable amounts of fluoride from fluoride-bearing water supplies. In addition, this child's permanent teeth were still free from dental caries when he was ten years of age. Unfortunately, this study only includes one child who was exposed to this means of fluoride administration, although two younger children in the same family are receiving the same level of fluoride from milk and are still caries-free as far as the still vital deciduous teeth are concerned. However, there is an interesting suggestion here that the human body can utilize an optimal level of fluoride from a foodstuff such as milk where there is a high level of calcium which would theoretically tend to depress its availability.

Earlier the senior author (A. E. Light, *Arch. Biochem. Biophys.* **47,** *477 (1953)*) had commented upon possible variations in fluoride intake between different individuals depending upon the relative amounts of milk and water ingested. In the interrogation of six subjects in Newburgh, New York, about the amount of water (including coffee, soup, soft drinks, but not citrus juices) and the amount of milk consumed during a pregnancy that had terminated nine months earlier, wide variations in fluid distribution were observed. If all other factors were constant in an area where the water contained fluorides, the women who consumed high milk and low water levels during pregnancy ingested less fluoride than the women with high water and low milk intakes.

However, the situation cannot be that simply defined because all other factors could not be constant except in the most unusual circumstances. Different food preferences could alter the fluoride intake of any woman appreciably from the expected average. In addition, no attention was made in this survey to the amount of fluoride introduced into vegetables and other foods by cooking in fluoridated water, nor to the influence of different cooking procedures. While none of these variations would be sufficient to increase the fluoride ingested to a toxic level, they could conceivably elevate to a perfectly satisfactory level the ingested amount in a woman who consumed little water and much milk.

The lack of detailed and accurate data on the amounts of fluoride available from sources other than water is clearly pointed up by these two papers. There is a clear need for the painstaking evaluation of these facets of fluoride metabolism.

VITAMIN B_{12} AND METHIONINE IN *E. Coli*

Various investigators have shown that several strains of *Escherichia coli* can utilize methionine sulfoxide, methionine sulfone or methionine sulfoximine in place of methionine (D. Mohlo and L. Mohlo-Lacroix, *Bull. Soc. Chim. Belges* **65,** *153* (*1956*); J. O. Lampen, M. J. Jones and C. B. Perkin *Arch. Biochem. Biophys.* **13,** *33* (*1947*); G. P. Wheeler, F. M. Schabel Jr. and H. E. Skipper (*Proc. Soc. Exp. Biol. Med.* **92,** *396* (*1956*)).

More recently, details of the activity of these compounds in *E. coli* strain B and mutants derived from this strain have been studied by L. S. Lockingen, R. M. Humphrey and O. Wyss (*J. Bacteriol.* **76,** *104* (*1958*)). Growth responses were tested on supplemented minimal plates by the methods described by E. B. Kalan and J. Ceithaml (*J. Bacteriol.* **68,** *293* (*1954*)). Inhibition data were obtained by use of liquid cultures in seven-inch test tubes which were incubated on a reciprocal shaker at 34° C.

Twenty-two mutants of *Escherichia coli* strain B were divided into three groups. The first group required methionine for growth, the second required either vitamin B_{12} or methionine, while the third group grew with homocysteine, homocystine or methionine. All these mutants were found to be perfectly capable of growth when methionine-sulfoxide was supplied as the sole substrate in glucose minimal medium. Since the sulfoxide also replaces homocysteine in methionine biosynthesis it is concluded that the entire molecule, including the sulfur and methyl group, is incorporated. T. L. Sourkes and T. Trano (*Arch. Biochem. Biophys.* **42,** *321* (*1953*)) have reported the reduction of methionine sulfoxide by *E. coli*.

None of the mutants of *E. coli* strain B grew with methyl methionine. Combinations of homocysteine with methyl methionine, S-methyl cysteine or dimethyl-β-propiothetin were without activity in the group I mutants. Methionine sulfoximine and methionine sulfone did not replace methionine in minimal medium for growth of these mutants, and, in fact, acted as growth inhibitors. The inhibition produced with low concentrations of these substances was partially relieved by glutamic acid, glutamine, methionine or methionine sulfoxide. Vitamin B_{12}, homocysteine, cystathionine and methyl methionine did not affect the inhibition.

It was further found that when cells of a vitamin B_{12}-stimulated mutant and a strict methionine-requiring mutant were starved and treated with T2R bacteriophage, both mutants demonstrated a small stimulation of bacteriophage production when the medium was supplemented with vitamin B_{12}. This phenomenon was thought to be due to limited methionine biosynthesis by these mutants.

These results indicate that *E. coli* strain B, in contrast to certain other strains, is incapable of utilizing certain substances, such as methyl methionine, methionine sulfoximine and methionine sulfone or of transforming these substances into methionine. Such studies should aid in elucidation of the mechanisms by which various derivatives of methionine may be converted, possibly with vitamin B_{12}, into methionine or other useful metabolites.

NOTES

American Board of Nutrition

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition, during the week of April 12–18, 1959, in Atlantic City, during the Federation meetings. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Robert E. Shank, Department of Preventive Medicine, Washington University School of Medicine, Euclid and Kingshighway, St. Louis, Missouri.

Food Quackery Exposed

The Committee on Nutrition Information of the Food and Nutrition Section of the American Public Health Association recommends to the attention of all who are interested in combatting food fads and nutritional quackery the new film entitled "The Medicine Man," which has been prepared by the American Medical Association in cooperation with the Food and Drug Administration and the National Better Business Bureau.

"The Medicine Man" represents a valuable and timely contribution to a concerted educational program to alert the public to the dangers of substituting food fads for sound nutrition.

Designed for use initially in local television programs, the 27-minute, black-and-white sound motion picture, exposes the dangers created by itinerant health lecturers who give misinformation on nutrition and by food supplement peddlers who misrepresent the nutritional value of their products. How the medical profession, food and drug administration, and voluntary agencies, such as the National Better Business Bureau, cooperate in fighting food quacks is also shown.

"The Medicine Man" is available through the A. M. A. Communications Division (535 North Dearborn Street, Chicago, Illinois) for showing to school, church, club and community audiences.

Nutritive Value of Cooked Meat

New information on the nutritive value of cooked meat is contained in a recent publication from the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater (R. M. Leverton and G. V. Odell, *Misc. Publ. MP-49 (1958)*).

This bulletin not only presents the nutrient content of cooked cuts of meat (beef, veal, pork and lamb) but also presents the nutrient content of individual portions of the cooked meat. Data are given on lean meat, lean plus marble, marble, and the fat. Cooking methods used were the usual household methods.

Analytical data are presented for caloric value, calcium, sodium, potassium and some of the B-vitamins (thiamine, riboflavin, niacin, pantothenic acid, vitamin B_6, and vitamin B_{12}).

The findings presented are considered a start toward supplying data from actual analyses for cooked lean and marbled meat with the separate fat removed since this is the way many people consume meat, avoiding the obvious fat portion. Figures in wide current use in calculating nutrient intakes from meats are based on the inclusion of considerable amounts of attached fat (B. K. Watt and A. L. Merrill, *U.S.D.A. Handbook No. 8 (1950)*).

Recent Nutrition Books

The Guinea Pig in Research. M. E. Reid, Ph.D. Laboratory of Nutrition and Endocrinology, National Institutes of Health, Bethesda 14, Maryland. Price $2.00.

Canadian Bulletin on Nutrition. L. B. Pett and G. F. Ogilvie. Published by Nutrition Division, Dept. of National Health and Welfare. Pp. 81. Price $1.00.

Living Resources of the Sea. (A Conservation Foundation Study). L. A. Walford. Published by the Ronald Press Co., New York. Pp. 321.

Domestic Food Consumption and Expenditure: 1956 Annual Report of the National Food Survey Committee. Published by Charles Birchall & Sons, Ltd., Liverpool 2, England. Price 9s. 6d. Net. Pp. 172.

Nutrients in Cooked Frozen Vegetables. L. J. Teply and P. H. Derse. A reprint from the Wisconsin Alumni Research Foundation, Madison, Wisconsin. Pp. 5.

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THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

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